PROTEOSTAT®

Protein refolding and aggregation sensing kit

Identify Optimal Protein Refolding Conditions and Maximize Recovery of Functional Protein

PROTEOSTAT® Protein refolding and aggregation sensing kit provides a concerted assay format employing a fractional factorial matrix design that facilitates screening of refolding parameters for a specific protein in different buffers, and a simple, homogenous assay format for monitoring protein aggregation using a proprietary red-emitting aggregation-sensitive molecular rotor dye. Selected samples with low fluorescence readout, thus low aggregation content, can then validated using traditional enzyme activity-based determination of refolding success.

Ordering Information

Order Online »

ENZ-51040-KP002	2x96 wells

Manuals, SDS & CofA

View Online »

- Don’t just refold - with integrated workflow, identify conditions that can cause off-pathway protein aggregation with PROTEOSTAT® dye
- Comprehensive set of optimized screening reagents and conditions that facilitate protein refolding.
- Rapid microplate screening using Design of Experiments (DOE)-valued approach to identify parameters that are critical to protein formulation.

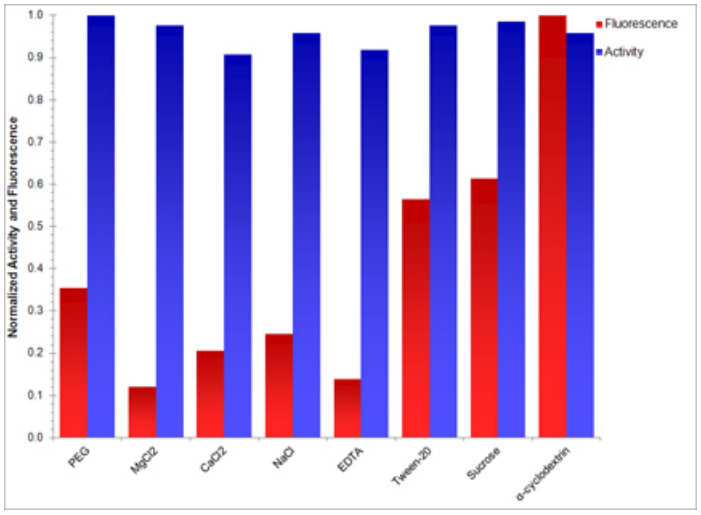

Figure 2. Refolding buffer 13B was determined to have a high activity and low fluorescence, as shown in Figure 1, and was selected to evaluate excipient effects on lysozyme refolding. Excipients were individually added into the refolding buffer with GSH/GSSG environment, and analyzed for aggregation and activity, as in Figure 1. The results indicated that $MgCl_2$ was the best at promoting refolding in refolding buffer 13B, with minimal tendency to aggregate.

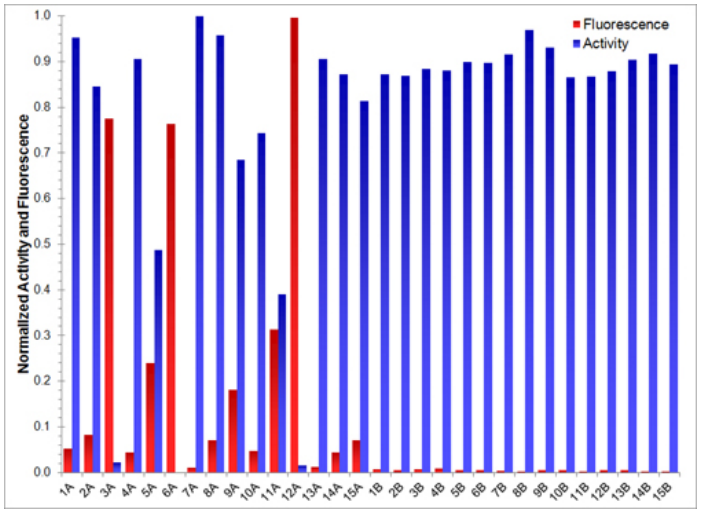

Figure 1. Refolding Lysozyme using a Redox Environment. The experiment was designed according the DOE matrix (in product insert, Table 1). In detail, 20 mg/mL lysozyme was first denatured in the 6M Guanidine solution at 4°C for 18 hours. Then, the denatured lysozyme was diluted 1: 20 into the various refolding buffers and incubated overnight at 4°C. For the PROTEOSTAT® Protein Refolding and Aggregate Sensing assay, a 1:20 dilution of the refolding solutions was added directly to PROTEOSTAT® Assay Buffer containing dye and fluorescence intensity was determined (Ex = 550 nm , Em = 610 nm). The enzymatic activity of the lysozyme in the refolding buffers was measured using 20 µg/mL Micrococcus lysodeikticus cell walls that had been excessively labeled with fluorescein, which caused dye quenching. When the lysozyme acts on this substrate, unquenched dye-labeled fragments of cell wall are released. The fluorescence intensity increase was determined (Ex/Em 490/520 nm). Native protein diluted in PROTEOSTAT® Assay Buffer was used as a reference for both the aggregation and activity assays. The refolding solutions with high aggregation signal typically had low enzymatic activity and thus should be avoided. 1A-15A: with DTT; 1B-15B: with GSH/GSSG.

Handling & Storage

Use/Stability With proper storage, the kit components are stable for one year from date of receipt.

Handling Protect from light. Avoid freeze/thaw cycles.

Long Term Storage -20°C

Shipping Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application Notes PROTEOSTAT® Protein refolding and aggregation sensing kit provides a fractional factorial matrix design to facilitate screening of refolding parameters for a specific protein in different buffers, and a simple, homogenous assay format for monitoring protein aggregation using a red-emitting aggregation-sensitive dye.

Contents

PROTEOSTAT® Detection Reagent, 22 µl
10X PROTEOSTAT® Assay Buffer, 1 x 2 ml
2X Refolding Buffers 1-15, 10 ml each
Lysozyme standard, 10 mg
Denaturant: 6M Guanidine Hydrochloride (GdnHCl), Tris-HCl (pH 8), 14 ml
100 mM DTT, 1 ml
GSH, 46.1 mg
GSSG, 18.4 mg
10 mM PEG, 1 ml
500 mM EDTA, 1 ml
400 mM $CaCl_2$, 1 ml
400 mM $MgCl_2$, 1 ml
1 M NaCl, 1 ml
0.1% Tween-20, 1 ml
1.2 M Sucrose, 1 ml
α-Cyclodextrin (200 mg/ml), 1 ml

Quality Control Using the procedure described in the manual, denatured lysozyme is refolded to compare the relative fluorescence in different buffers.

Quantity For 192 reactions

Technical Info / Product Notes The PROTEOSTAT® Protein refolding and aggregation sensing kit is a member of the PROTEOSTAT® product line, reagents and assay kits that have been extensively tested in protein aggregation applications. PROTEOSTAT® reagents and kits are optimal for demanding protein analyses involving aggregation using microscopy, flow cytometry, microplate readers and HCS/HTS, where consistency and reproducibility are required.